ANTIMICROBIAL AND ANTIBIOFILM ACTIVITIES OF MEDICINAL PLANTS EXTRACTS AGAINST MULTI-DRUG RESISTANT UROPATHOGENS

A synopsis is submitted to

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For

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1. Abstract

Urinary tract infections (UTIs) are becoming more challenging in the recent era due to their high recurrence rates and resistance to frontline antibiotics. Uropathogenic bacteria and mostly *E. coli* account for the 90% of all UTIs. The Uropathogens possess various virulence factors that contribute for their effective colonization, higher persistence and pathogenesis of the disease. Biofilm formation ability of these pathogens is mainly linked to nosocomial infection and particularly catheter associated UTIs. Current microbial inhibition strategies are based on planktonic bacterial physiology and known to have limited efficiency on the growth of biofilm communities. This problem is further intensified by the emergence of increasingly resistant strains. At present more attention is given to isolation of nontoxic bioactive compounds capable of inhibiting biofilms. One of the attractive bioresources is medicinal plants based novel bioactive molecules. Medicinal plants are known for their potential to produce antibiotics, enzymes, enzyme inhibitors and other pharmaceutical agents. Several reports revealed that many actinomycetes produce antibiofilm agents against different pathogenic organisms. However, the efficiency of these antimicrobial agents is currently decreasing due to emergence of multi drug resistant pathogens. In addition to this very few reports are found against uropathogenic biofilm forming organism. Therefore, it is important to search for alternative therapeutics to control biofilm associated uropathogenic infections. In the present study we propose to characterize the bioactive metabolites produced by medicinal plants and examine it's role in inhibition or dispersion of biofilms formed by uropathogens.

2. Introduction

Infectious diseases caused by microorganisms are among the leading causes of death worldwide, particularly in developing countries. Bacterial infections occur when the host's defense cannot withstand invading bacteria. Urinary tract infection (UTI) is the second most common microbial infection in humans (Foxman, 2014., Francesko et al., 2016), affecting around 150 million people each year globally. It poses a heavy economic and public health burden with estimated cost in around six billion dollars for treatment and healthcare (Stamm and Norrby, 2001). Bacterial urinary tract infections affect all age groups including young women, children, and the elderly. It is estimated that approximately 40% of women have had a UTI at some time in their lives. They may occur in any part of the urinary tract and being more common in diabetic patients. Diabetic cystopathy and micro-vascular diseases may cause changes in host defense mechanism of kidney leading to more prevalence of UTI (Sridhar et al., 2002). UTI patients are susceptible to

cause emphysematous cystitis, pyelonephritis, renal or perinephric abscess, bacteremia, and renal papillary necrosis. Bacteraemic patients have more chance to develop acute renal failure (Ronald and Ludwig, 2001).The gram-negative bacteria play an important role in UTI and the most common causative agent is *Escherichia coli* being found in 75–90% cases (Karlowsky et al., 2002; Nakhjavani et al., 2007).The other bacterial uropathogens are *klebsiella* sp., *Proteus mirabilis*, *Pseudomonasaeruginosa*, *Enterococcus* and coagulase negative *Staphylococci* (Omigieet al., 2009).

UTIs are treated through a diverse group of antibiotics; betalactams, fluoroquinolones, aminoglycosides, and others. However, uropathogens are becoming drug-resistant, which has become a hindrance to the disease's control. The reasons for drug resistance can be spontaneous mutation or acquisition of resistant genes through horizontal transfer that often occurs in organisms of different taxonomy. The bacterial resistance to antibiotic is significant to medical industry and global threat in the treatment of urinary tract infections (UTIs) and hence a constant topic of discussion across the world. It greatly alarmed that the incidence of multidrug resistance (MDR) development and infection are increasing in UTIs (Shahi and Kumar, 2016). Uropathogens are one of the most important MDR bacteria and it developed resistance against almost all commercially available antibiotics including Ampicilin, amoxicillin, ciproflaxacin and all third generation cephalosporin due to the co-expressed resistance mechanisms that lead to MDR effect in these pathogens. Treatment of such MDR is also a major topic of concern since the pathogens are almost resistant to current antibiotics and they easily develop resistance to the new drugs also (Rajivgandhi et al., 2014; Rajivgandhi et al., 2018). In particular, Gram negative bacteria play a vital role in UTIs mainly due to synthesizing some enzymes, efflux pump, and target site modification (Kelly et al., 2016). Recently MDR in these bacterial uropathogens is found to increase significantly and they show resistance to at least one drug commonly used for eradication and few are resistant to all approved antibiotics (Paterson, 2007). It causes recurrent infections (40-75%) in UTIs due to improper usage, un prescribed format of antibiotics, over dosage and long-term treatment in human, agro farms and veterinary (Moges et al., 2014).

Biofilm development can be considered as a virulence determinant responsible for the long-lasting persistence of bacteria in the genitourinary tract. Urinary catheters and any other prosthetic devices predispose to UTI by destroying natural barriers and providing a site for infection by serving as a substrate for biofilm formation. Fundamental research studies have demonstrated that biofilm cells are more resistant to antimicrobial agents than planktonic bacterial

cells (Tabibian et al., 2008). Reduced antibiotic susceptibility of biofilms contributes to the persistence of infections, such as those associated with implanted devices. Poor antibiotic penetration, nutrient limitation, slow growth, and the formation of persistent cells are hypothesized to constitute multi-layered biofilm (Stewart, 2002). Conventional antimicrobials are not effective against biofilms, and there are relatively few novel compounds or strategies under development or under clinical testing. Increased knowledge regarding the formation of biofilms has led to identification of several possible points for targeted antibiofilm approaches (Flemming and Wingender, 2010; Römling and Balsalobre, 2012).

The problem of antimicrobial resistance is recognized as a global public health concern that provokes high financial costs to healthcare systems and is fueled by the overuse of antibiotics. Since 2014 World Health Organization has highlighted that antibiotic resistance in uropathogens is a key pressure point in the global antimicrobial resistance crisis. Therefore, there is an urgent need to understand UTI pathophysiology better and develop innovative products that use non-antibiotic strategies to prevent and treat UTI.

Since ancient times, medicinal plants are alternative resources used by the population for therapeutic purposes. It is estimated that approximately 90% of people have already used natural medicine for the primary relief of some discomfort caused by diseases. The World Health Organization (WHO) encourages research regarding the use of plants, with the aim of ensuring that the therapeutic properties present in these natural products can be applied to prevent/treat diseases without damaging health. From the ancient period, plants and their parts have been used as a source of medicine to treat different diseases. Ethno medicine is still considered a primary source of treatment in many communities. About 60% of the world's populations, with 80% in developing countries, are directly dependent on medicinal plants for treatment of different diseases. The known antimicrobial properties of both plant extracts and phytochemicals can be of great significance in developing new therapeutic modalities in the fight against emerging multiple drug resistance among bacteria. The World Health Organization (WHO) also encourages research regarding the use of plants, with the aim of ensuring that the therapeutic properties present in these natural products can be applied to prevent/treat diseases without damaging health.

The antimicrobial effects of plant extracts are attributed to multiple mechanisms: directly killing microbes, interfering with microbial adhesion to epithelial cells and biofilm formation, stopping the multiplication of pathogens, restoring dysbiosis, improving the host protection acting on natural barriers, serving as immunomodulators, or boosting body oxidant status (Belkaid and

Hand., 2014). Based on this, the present study is proposed to evaluate the potential of plant extracts and phytochemicals on growth and biofilm formation in multidrug-resistant uropathogens.

3. Review of literature

Urinary tract infections (UTIs) are serious health affecting problems worldwide. *E.coli* accounts for approximately 85% of community acquired UTIs and 50% of hospital acquired UTIs (Ramakant k et.al, 2011). According to (Iqbal et al, 2010), Urinary catheters destroy natural barriers and provide a nidus for infection by serving as a substrate for biofilm formation. Several studies have demonstrated that biofilm cells are more resistant to antimicrobial agents than planktonic bacterial cells (Tabibian J.H et.al, 2008). The resistance of biofilms to antibiotics contributes to the persistence of infections, such as those associated with implanted devices .Bacterial biofilms play an important role in UTIs, responsible for persistent infections leading to recurrences and relapses (Waturangi. et al, 2016). Approximately 40% of women have had a UTI at some time in their lives (Kunin CM, 2014). *Escherichia coli* (*E. coli*) is the most frequent agent (about 80%) of UTIs in humans and one of the most common causes of Gram-negative nosocomial infections. Further, other bacteria such as *Proteus mirabilis, Klebsiellapneumoniae, Pseudomonasaeruginosa*, *Enterococcus* spp., *Enterobacter* spp , group B *Streptococcus,* and *Staphylococcus saprophyticus* are also involved (Foxman B, 2002).

Bacterial forming Biofilms eradication is difficult due to the high level of antimicrobial resistance showed by these structures. Compounds with antibiofilm properties are necessary in order to prevent the formation and disrupt the development of biofilm. The ideal situation is a compound or a group of compounds that present not only antibiofilm activity but also the capacity to eradicate multidrug-resistant bacteria. It is also important to study the antibiofilm activity of new combinations of therapeutic strategies including new ones and old antibiotics. Virulence factors should be considered important targets for developing antivirulence compounds that may be applied in the prevention and treatment of infectious diseases.(Sara M. Soto, 2014).

Extracts from *Betula pendula, Equisetum arvense*, *Herniaria glabra, Galium odoratum*, *Urtica dioica,* and *Vaccinium vitis*idea had influenced effect on bacterial survival and virulence factors involved in tissue colonization and biofilm formation of the uropathogenic *Escherichia coli* (Wojnicz et al ; 2012).

Leme et al were reported that the roots of *Cochlospermum regium* showed reduction of the biofilm formation for *E. coli* and *C. tropicalis* and antimicrobial action at 1 mg/mL and 0.5 mg/mL, respectively. The extract showed no cytotoxicity and mutagenicity at the concentrations tested.

This study demonstrated that *C. regium* leaves are a viable option for the treatment of genitourinary infections and for the species preservation (Leme et al ; 2017) . In addition to this, Pomegranate (*Punica granatum* L.), black chokeberry (*Aronia melanocarpa* Michx.), and cornelian cherry (*Cornus mas* L.) have great potential to be used for prevention or in a combined antibiotic therapy to cure UTI (Tache et al; 2022). *Holarrhena floribunda* stem bark extract has strong biofilm formation inhibition properties, which could be attributed to the presence of steroidal alkaloids (Ampofo et al; 2020).

In other study which is reported that extracts obtained from *C.englerianum* and *E. depauperate* showed more potent antibacterial activity on MRSA and *Enterococcus faecalis* with IZ 25 and 27 mm respectively. *E. coli* and *K. pneumoniae* were inhibited by those extracts with IZ ranging 9–25 mm and 11–27 mm respectively. *E. faecalis* and *K. pneumoniae* were more susceptible bacteria to the respective extracts (Gadisa & Tadesse ; 2021).

4. Objectives of Research

- Collection of medicinal plants from Nanded and nearby region.
- Preparation of various extracts from selected medicinal plant parts
- Screening and evaluation of medicinal plant extracts for antimicrobial and antibiofilm activities against uropathogens.
- Qualitative and quantitative phytochemical analysis of selected plants extracts
- Determination of MIC, MBC and MFC of plant extracts against growth of uropathogens
- Characterization of potent bioactive extracts.
- Quantification of antibiofilm activity of potent bioactive extracts against biofilm forming uropathogens.

5. Methodology to be adopted

1. Plants known for medicinal importance will be collected from Nanded and nearby regions of Marathwada. The collected plants will be identified based on their taxonomic features.

2. Bioactive plant extracts will be prepared by using various extraction methods as maceration, infusion or solvent extraction.
3. Uropathogens will be isolated from sample collected from patients with history of UTI and identified using biochemical and molecular characterization.
4. Drug resistance pattern among isolated uropathogens will be studied using standard and commercially available antibiotics used for treatment of UTI. Agar well diffusion or disc diffusion methods will be used.
5. Biofilm formation potential of uropathogens will be determined using standard methods such as Congo red assay and microtitre plate methods.
6. Screening of medicinal plants extracts for antibacterial activity against drug resistant uropathogens using agar well diffusion or broth dilution methods. MIC, MBC and MFC values for potent extracts will be determined.
7. Screening of medicinal plants for antibiofilm activity against drug resistant uropathogens using standard methods.
8. The selected plant extracts will be used in synergy with each other based on their potency or with standard antibiotics to evaluate their efficacy against drug resistant and biofilm forming uropathogens. Checker board assay will be used for studies on synergy.
9. Characterization of bioactive metabolite from selected plant extract will be carried out using standard analytical methods such as HPTLC, GC-MS, FTIR and NMR.

6. Importance of Study

- **International status:**

Urinary Tract Infection (UTI) is one of the most common infections caused by a range of bacterial pathogens, mainly *Escherichia coli*, *Klebsiella*, *Enterococcus*, and *Staphylococcus*. Women are more vulnerable to UTIs than men, with the risk increasing with age (Medina and E. Castillo-Pino., 2019). According to a study in Kathmandu Medical College and Teaching Hospital, Duwakot, Nepal, by Pradhan & Pradhan (2017), 75% of UTI patients were female. Up to 50% of women have at least one symptomatic UTI in their lifetime, and the risk of infection increases in postmenopausal women.

Dikshanta et al., (2021) reported antimicrobial activity of *Ocimum sanctum, Asparagus officinalis*, and *Tribulus terrestris*, along with actinomycetes isolated from the root vicinity of the plants against three multi-drug resistant uropathogens. All three plant extracts inhibited

Escherichia coli. Only *A. officinalis* exhibited effect over *Klebsiella oxytoca*. Extract of *O. sanctum* and *T. terrestris* prevented the growth of *Staphylococcus aureus*. *A. officinalis* was more effective against Gram-negative pathogens, and *O. sanctum* was more effective against Grampositive pathogens.

Coriander oil has been described in the past to be effective against Gram-negative (E. coli, Salmonella typhi, Klebsiella pneumonia, and Proteus mirabilis) and Gram-positive bacteria (Bacillus megaterium, S. aureus, and Bacillus sp.) (Lo Cantore et al.,2004; Matasyoh et al., 2009).

Dorota Wojnicz et al (2012) focused on medicinal plant species used in UTI Prevention. In this study influence of *Betula pendula, Equisetum arvense, Hemiaria glabra, Urtica dioria* & *Vaccinium vitis idaega* extracts on bacterial survival and virulence factors in biofilm formation of uropathogenic *E. coli* rods. The extracts of *H. glabra* and *V. vitisidaea* showed highest growth inhibitory effects.

Pomegranate (*Punica granatum* L.), black chokeberry (*Aronia melanocarpa* Michx.), and cornelian cherry (*Cornus mas* L.) have great potential to be used for prevention or in a combined antibiotic therapy to cure UTI, but more studies and clinical trials in specific population groups are required (Tache et al, 2022). Gadisa and Tadesse (2021) carried out a study to evaluate the antimicrobial effect and screening phytochemical potential of *Rumex abyssinicus, Cucumis pustulatus, Discopodium penninervium, Lippia adoensis, Euphorbia depauperata*, and *Cirsium englerianum a*gainst drug resistant microbes. They found out that extracts obtained from *C.englerianum* and *E. depauperate* showed more potent antibacterial activity on MRSA and *Enterococcus faecalis* with IZ 25 and 27 mm respectively. *E. coli* and *K. pneumoniae* were inhibited by those extracts with IZ ranging 9–25 mm and 11–27 mm respectively. *E.faecalis* and *K. pneumoniae* were more susceptible bacteria to the respective extracts. *R. abyssinicus* showed promising antifungal effect with had 21 mm IZ and MIC range 16-32 μg/ml on *C.albicans*.

- **National status**

Chaudhary et al., (2017) evaluated the potential of some medicinal plants used in Ayurveda in treating multiple drug-resistant human pathogens causing urinary tract infections (UTIs). This

study concluded that the medicinal plants used in Ayurveda to treat UTIs are effective against multiple drug-resistant uropathogens.

Seventeen Indian folklore medicinal plants were investigated to evaluate antibacterial activity of aqueous, ethanol and acetone extracts against 66 multidrug resistant isolates of major urinary tract pathogens (*Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*) by disc diffusion method. Ethanol extract of *Zingiber officinale* and *Punica granatum* showed strong antibacterial activity against *Escherichia coli*. Ethanol extracts of *Terminalia chebula* and *Ocimum sanctum* exhibited antibacterial activity against *Klebsiella pneumoniae*. Ethanol extract of *Cinnamomum cassia* showed maximum antibacterial activity against *Pseudomonas aeruginosa* while ethanol extract of *Azadirachta indica* and *Ocimum sanctum* exhibited antibacterial activity against *Enterococcus faecalis*. The results support the folkloric use of these plants in the treatment of urinary tract infections by the tribals of Mahakoshal region of central India (Sharma et.al.,2009).

Mishra et al (2021) examined effectivity of 9 tropical flowering plants (*Anogeissus acuminata, Azadirachta indica, Bauhinia variegata, Boerhaavia diffusa, Punica grana-tum, Soymida febrifuga, Terminalia chebula, Tinospora cordifolia* and *Tribulus terrestris*) for possible use as source of antimicrobials for multidrug resistant (MDR) bacteria, along with main-stream antibiotics. It was found that only *E. coli* were susceptible to methanolic extracts of V. negundo L. Methanolic extract of *E. cardamomum* (L.) showed significant antibacterial activity against *P. aeruginosa* and some activity against *S. aureus*.

In other study 3 plant extracts (*A. officinalis,O. sanctum, and T. terrestris*), were showed inhibition on *Escherichia coli*. Only *A. officinalis* exhibited effect over *Klebsiella oxytoca*. Extract of *O. sanctum* and *T. terrestris* prevented the growth of *Staphylococcus aureus*. *A. officinalis* was more effective against Gram-negative pathogens, and *O. sanctum* was more effective against Gram positive pathogen (Luitel et al, 2021).

7. **Proposed work Plan/ Formulation and Structure of Study:**

Year-wise Plan of work and targets to be achieved

- **First year (2021-2022):-**

- Completion of course work and Literature survey
- Collection and identification of medicinal plants
- Isolation of Uropathogens

- **Second year (2022-2023): -**

- Testing drug resistance and biofilm formation potential of uropathogens
- Preparation of extracts from selected medicinal plants
- Screening of medicinal plant extracts for antimicrobial and antibiofilm activity

- **Third year (2023-2024): -**

- Characterization of potent bioactive extracts
- Preparation and submission of research articles to the reputed national and international journal.
- Compilation and analysis of data
- Submission of thesis to the university

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